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ORIGINAL MEMOIRS.

AN EXPERIMENTAL AND HISTOLOGICAL STUDY OF CARGILE MEMBRANE.[1]

WITH REFERENCE TO (1) ITS EFFICACY IN PREVENTING ADHESIONS IN THE ABDOMINAL AND CRANIAL CAVITIES AND AROUND NERVES AND TENDONS, AND (2) ITS ULTIMATE FATE IN THE TISSUES.

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EXPERIMENTAL STUDY BY DR. CRAIG.

IT is not worth while to attempt an enumeration of the efforts which have been made to prevent adhesions in the peritoneal cavity; nor is it necessary to lay stress upon the necessity for preventing such adhesions when it can be accom-

[1] Read before the Philadelphia Academy of Surgery, February 6, 1905.

[2] The death of Dr. Craig, March 14, 1905, from cerebrospinal meningitis, contracted while in attendance upon a patient suffering from that disease, lends additional interest to this report of his latest study.

plished. Adhesions within this cavity are, on the whole, beneficial, however harmful under certain circumstances. We cannot expect Nature to differentiate; it is left to the devices of the surgeon to prevent, if he can, adhesions when they would be harmful, and, in truth, it may be stated that he has succeeded but poorly.

In May, 1902 (*Medical Record*, May 17, 1902), Dr. Robert T. Morris, of New York, published the results of a series of experiments carried out upon rabbits, to determine the value of a specially prepared animal membrane derived from the peritoneum of the ox. The effort was made to prevent adhesions within the peritoneal cavity. Attention was first called to this membrane by Dr. Charles Cargile, of Bentonville, Arkansas, who sent Dr. Morris specimens of the membrane to be used, hence the New York surgeon termed the material Cargile membrane, an eponym which has since become common. The membrane is not essentially different from goldbeaters' skin, except in the method of its preparation. As prepared by Johnson and Johnson, it comes in small sheets about eight by sixteen centimetres in size, and is treated after a special method. Some of it is treated evidently somewhat after the manner of chromicized catgut, and is hence termed chromicized Cargile membrane; another preparation is unchromicized. The report of Dr. Morris, on the whole, was favorable, and he appeared to believe that the membrane possessed distinct advantages in preventing adhesions. His conclusions, somewhat abridged, were as follows: Cargile membrane seems to resist absorption in the peritoneal cavity for more than ten days and less than thirty days. Its presence apparently causes the formation of temporary loose adhesions, which are harmless, and which become absorbed for the most part in less than thirty days. The membrane seems to cause very little disturbance to the peritoneum; it does not furnish a good culture medium for bacteria, and it protects areas of peritoneal surface that have suffered injury to their endothelial covering, until new endothelial cells have repaired the injury without involving the neighboring peritoneum. It is not

necessary to suture the membrane in place, as it becomes instantly adherent to moist surfaces, and is not readily dislodged afterwards.

If the membrane possessed the merits which Dr. Morris's experiments seemed to warrant, I could not understand why surgeons did not make more general use of it as a protective covering for surfaces denuded of peritoneum, and in other situations in which it would appear applicable.

To satisfy myself of the value, or the reverse, of this membrane, in the summer of 1904 I undertook a series of experiments in the laboratories of the Jefferson Medical College Hospital. The membrane was kindly furnished me by the manufacturers above mentioned. Dogs were used in the experiments, and not only were tests made in the peritoneal cavity, but likewise in the protection of tendons and nerves, and the cranial cavity was invaded. After various intervals of time the seat of operation was exposed in each case, the clinical conditions ascertained, and, in a number of instances, specimens of tissue which had been in contact with the membrane were submitted to Dr. Ellis, associate in pathology at the College, who kindly undertook the microscopic investigations in this research. His findings are set forth in a separate portion of this paper.

It will be noted in the following recitation of the experiments, that when Cargile membrane was used in the peritoneal cavity of a dog, in most instances the membrane was anchored in place by fine silk sutures. It was of course recognized that the irritation produced by the sutures would, in a measure, vitiate the experiment; but it was believed, and subsequent experiments showed the assumption warranted, that if a sufficiently large piece of membrane were used, so that the suture could be placed on either side of the intestine well towards the mesenteric attachment, the irritation produced by them need not interfere with the surface opposite that attachment. Furthermore, it was found by simple tests that the statement of Dr. Morris, namely, that the membrane would adhere readily and sufficiently to a denuded surface without

suturing, was correct so long as the peritoneal, or denuded surface, was dry, or relatively so; but directly the intestine with the attached membrane was returned to the peritoneal cavity and bathed for a short time in the peritoneal fluid, the membrane ceased to adhere and readily slipped from the particular point covered. With this fact, repeatedly demonstrated, in mind, we of course could not expect that the membrane would adhere and remain where placed, despite the various movements of the animal and the peristaltic activity of the abdominal viscera. I therefore anchored the membrane by sutures, to be sure that it remained *in situ* over the denuded area. It is conceivable that Cargile membrane may be placed in the pelvis or similar situations, between the peritoneum and a denuded surface, or between denuded surfaces, and remain in place without being anchored. It certainly will not remain, when unanchored, on either visceral or parietal surfaces when these are bathed in fluids and subjected to friction, be it never so little, from peristaltic activity. I may state in passing that I tried anchoring by means of celloidin and also by means of formalin-gelatin. Neither was a success.

All the dogs operated upon were profoundly anæsthetized with ether and treated according to the rules of aseptic surgery, so far as could be conveniently carried out. In only one instance did peritonitis occur, and this was from a defective end-to-end anastomosis.

EXPERIMENT No. I.—The abdominal cavity of a dog having been opened, a loop of intestine near the stomach was lifted out and two surfaces opposite the mesenteric attachment, each one and one-half centimetres square, were denuded of peritoneum, sponged until dry, covered with separate pieces of unchromicized Cargile membrane without anchoring, the abdominal wound being closed with silkworm-gut sutures. Twelve days later the abdomen was reopened and a mass of omentum was fairly firmly adherent to the distal denuded surface, and both omentum and liver were adherent to the proximal denuded surface; no Cargile membrane was found; either it did not remain *in situ*, or it had been absorbed and adhesions formed subsequently.

EXPERIMENT No. II.—The abdomen of a dog was opened and a loop of small intestine was brought out. A surface one centimetre by one and one-half centimetres was denuded of peritoneum and covered with

chromicized membrane, the piece being large enough to extend back on either side to the mesenteric attachment, where it was anchored by sutures. Ten centimetres distal to this was anchored in like manner a piece of unchromicized membrane over an undenuded surface; further distal by ten centimetres was anchored similarly, a piece of chromicized membrane over a denuded surface; while still distal to this was anchored a sheet of chromicized membrane over an undenuded surface. In this experiment I sought to compare the effects of placing chromicized and unchromicized membrane each on denuded and undenuded surfaces. The sutures were so placed that I could identify the several pieces. Fourteen days later, forty centimetres of the bowel containing the four separate experiments were resected, examined macroscopically, and submitted to Dr. Ellis for microscopic examination. It was of interest to note that while a mass of adherent omentum completely covered the site of operation in each case, and a loop of bowel was adherent in two places, yet the Cargile membrane was at no place completely absorbed; both the chromicized and unchromicized membranes were clearly detected by splitting the mass of adherent omentum. The latter was adherent directly to the membrane, and more firmly still to the bowel at the periphery of the membrane. Under the membrane the denuded area was rough and scar-like, and there was no macroscopic evidence of regenerating peritoneum. Clearly, used in this manner, the membrane would not prevent adhesions.

EXPERIMENT No. III.—The abdomen of a dog was opened and two areas of the duodenum seven centimetres apart and each one and one-half by two centimetres in area were denuded of peritoneum, and the proximal one was covered with unchromicized Cargile membrane, while the distal one was left with its raw surface exposed. At the same operation an area two centimetres square on the anterior surface of the stomach was denuded of peritoneum and covered similarly with unchromicized membrane, but the latter was not anchored by suture. The abdominal wound was closed in the usual way. Nineteen days later the abdomen was reopened and firm adhesions were found at each site of denudation. Apparently they were as firm and as numerous where the Cargile membrane had been placed as where it had not been placed. A careful search revealed no Cargile membrane.

EXPERIMENT No. IV.—The abdomen of a dog was opened and four pieces of Cargile membrane were placed as follows: (*a*) A piece of unchromicized membrane was placed over a denuded surface one by two centimetres in size and anchored well towards the mesenteric attachment; (*b*) a piece of unchromicized membrane was placed over an undenuded surface and similarly anchored; (*c*) a piece of chromicized membrane was placed over a denuded surface of similar size and anchored, as above; and (*d*) a piece of chromicized membrane was placed over an undenuded surface and attached by sutures as in the foregoing. The number of sutures differed with each piece anchored, so that the several pieces could be recognized. Four days later the abdomen was again opened and thirty-

five centimetres (fourteen inches) of the bowel, to which the four pieces of membrane had been attached, was resected and end-to-end anastomosis done. Adherent omentum completely covered and surrounded every piece of membrane. The adhesions were easily broken up, being so recent, but they were numerous. At the two places where the unchromicized membrane was placed, none of the Cargile membrane could be found macroscopically, though Dr. Ellis was able to find fragments microscopically. The chromicized membrane, however, was plainly visible where it had been placed. Neither had prevented adhesions, particularly at the periphery of the membrane. The resected portion of the intestine was submitted to Dr. Ellis for microscopic examination.

EXPERIMENT No. V.—A dog's abdomen was opened and an area one and one-half by four centimetres was denuded of peritoneum and covered with unchromicized membrane. It was anchored *in situ* as above explained. Ten centimetres distal to this, an area one and one-half by two centimetres was similarly denuded, but left exposed without Cargile covering. The abdomen was closed and sixteen days later reopened. A mass of omentum covered the entire site of operation in each instance, and no membrane was found.

EXPERIMENT No. VI.—A dog's abdomen was opened and a surface one and one-half by three centimetres on the duodenum was denuded of peritoneum and covered with the unchromicized membrane, the edges being anchored as in previous instances. Ten centimetres distal to this a similar area was denuded and not covered with membrane. Eleven days later the abdomen was reopened and fairly firm adhesive omentum covered alike both areas. No membrane was found.

EXPERIMENT No. VII.—A dog's abdomen having been opened, an area one and one-half by two centimetres on the duodenum was denuded of peritoneum and covered with unchromicized Cargile membrane, the latter being anchored as above. Three days later the abdomen was reopened. A large omental graft had covered the entire site of operation. The membrane immediately covering the actual denudation had disappeared, but it persisted in the rest of its extent; that is, the centre of the sheet of membrane had been digested or dissolved by the raw surface. This showed that some element in the actual wound acted, probably in a digestive capacity, in dissolving the membrane in immediate contact. A portion of the intestine containing the field of operation was resected and submitted for microscopic examination.

EXPERIMENT No. VIII.—A dog's abdomen having been opened, a small area of duodenum was denuded of peritoneum, covered with unchromicized membrane which was anchored by sutures, and this in turn was covered by a piece of sterile rubber dam which extended well beyond the Cargile membrane; this, too, was in turn anchored by suture. Three days later the abdomen was reopened, and it was found that a mass of omentum and aplastic lymph completely covered the entire site of operation, including the rubber dam. I desired by this experiment to determine whether it was a phagedenic property of the omentum that

destroyed the membrane, or was it granulation tissue, or was it peritoneal fluid? The mass was removed from the rubber dam; the latter was likewise carefully removed and no Cargile membrane was recognized macroscopically, though fragments were observed by Dr. Ellis microscopically.

EXPERIMENT No. IX.—This was a repetition of Experiment VIII, except that the abdomen was reopened on the sixth day instead of the third after operation. Practically, the same conditions were found, namely, the sheet of rubber dam, under which the Cargile membrane had been placed, was covered with an omental graft, and on examination the Cargile membrane had all disappeared to macroscopic view, though seen by Dr. Ellis microscopically.

EXPERIMENT No. X.—Experiments VIII and IX appeared to offer fair evidence that it was not the omentum *per se* that had destroyed the membrane, but it proved nothing as to the action of the peritoneal fluid. Accordingly, I placed a piece of unchromicized membrane, five centimetres square and made into a small roll, in a glass tube one centimetre in diameter and seven and one-half centimetres long, and containing about a dozen small perforations; in another tube of about equal size was placed a similar piece of the chromicized variety. These tubes were closed sufficiently to prevent the escape of the membrane and placed loose in the peritoneal cavity of a dog. Fourteen days later the abdomen was reopened and both tubes were easily found. The tube containing the chromicized membrane was practically free, and when removed the membrane was quite softened, pale, and œdematous, but apparently little changed in other respects. It was delivered to Dr. Ellis for further examination. The tube which had contained the unchromicized membrane was wrapped about with omentum, but the membrane had entirely disappeared, leaving the tube empty. Clearly, the chromicized membrane was much the more resistant.

EXPERIMENT No. XI.—From the glass-tube experiments we had fair proof that the unchromicized membrane would soon disappear when placed in the abdominal cavity, without actual contact with the omentum. It appeared a natural deduction that the peritoneal fluid could itself be potent in dissolving the membrane. To exclude the phagocytic action of the leucocytes, at Professor Coplin's suggestion and under his direction, I placed a piece of unchromicized membrane three centimetres square in a celloidin capsule five centimetres long, containing salt solution. Pathologists state that this capsule will permit the osmosis of the body fluids, but leucocytes will not pass through its wall. The sealed capsule, with the contained membrane, was placed free in the abdominal cavity of a dog. On the seventh day the capsule was removed and opened. The membrane, aside from being œdematous and apparently thickened, was little changed macroscopically. There was little fluid left in the capsule. The membrane was submitted to Dr. Ellis.

EXPERIMENT No. XII.—The above experiment was repeated in every detail, except the celloidin capsule was not removed until the thirtieth day. It was easily found wrapped in a small mass of omentum. There was apparently no infection. Professor Coplin opened the capsule. It

contained a milky, slightly blood-stained fluid, and the membrane, hardly recognizable as such, just at the point of disintegration. It had apparently almost dissolved. Professor Coplin examined some of the fresh fluid from the capsule under the microscope. The findings are detailed in the paper of Dr. Ellis.

EXPERIMENT No. XIII.—The left tendo-Achillis and the left posterior tibial nerve of a dog were exposed, and each was wrapped separately with four turns of unchromicized Cargile membrane. At the same operation the right tendo-Achillis and accompanying posterior tibial nerve were exposed and wrapped with three turns of chromicized Cargile membrane. The wounds were sutured. Fourteen days later the dog was killed and three centimetres of each tendon and its accompanying nerve were resected *en masse*. Examined macroscopically, the right nerve, around which the chromicized membrane had been placed, showed the membrane still in place; and while there was a mass of granulation tissue outside the membrane, the latter had plainly protected the nerves, there being no macroscopic adhesions to the latter whatever, except at either end of the tube formed by the protecting Cargile membrane. The left nerve, about which the unchromicized membrane had been placed, showed no Cargile membrane macroscopically, though microscopic fragments were found by Dr. Ellis. And while adhesions to the nerve were distinctly fewer and less firm where it had been protected by the membrane than where it had not, yet fairly firm adhesions (for fourteen days) were present, and it was evident that the nerve had not been so well protected as where the chromicized membrane had been employed.

EXPERIMENT No. XIV.—Under ether the two tendons, as above mentioned, of a dog were exposed for a distance of five centimetres, and when each tendon was raised from its bed it was wrapped about by two turns of unchromicized membrane and the skin wound was closed. Twenty days later the dog was killed, and both tendons and the accompanying posterior tibial nerves were removed. Plainly, there were fairly firm adhesions to the tendon, more marked than at points not subjected to trauma. No membrane was found. The specimens were submitted to Dr. Ellis.

EXPERIMENT No. XV.—The right tendo-Achillis of a dog was exposed, lifted from its bed, and four turns of unchromicized Cargile membrane were passed around it, thus isolating it completely. The accompanying posterior tibial nerve was isolated, wrapped separately with two turns of membrane, and plaster dressing was applied to the dog's leg. It was hoped by immobilizing the parts that a better idea of the actual protection afforded by the membrane, if any, could be had. Inability to keep the wound aseptic necessitated the removal of the plaster dressing. Five days after operation the wound was reopened. A mass of granulation tissue surrounded the tendon and nerve, but not a vestige of Cargile membrane could be found. Plainly, it had afforded little or no protection. It could only be assumed that the granulation tissue would follow the usual course and result in scar tissue, thus causing adhesion, unless constant motion prevented.

EXPERIMENT No. XVI was a repetition of Experiment XV, except that, in addition to covering the nerve and tendon separately, a piece of Cargile membrane two and one-half by five centimetres in dimensions was made into a small roll wrapped about with fine silk thread by a number of turns and placed in the depth of the wound between the nerve and tendon. Nine days later the wound was reopened, and, while granulation and organizing tissue was plentiful, no Cargile membrane was found, not even the roll mentioned above, but the rolled-up silk ligature was easily found. Evidently the membrane had all been dissolved.

EXPERIMENT No. XVII.—The right tendo-Achillis and right posterior tibial nerve were exposed and wrapped separately with two turns of unchromicized Cargile membrane. The left side was treated in like manner, and the wound closed. On the fifty-fourth day after operation the dog was killed and each tendon and nerve was resected and examined. With the exception of a very small amount of scar tissue about the tendons and nerves, they appeared normal. No Cargile membrane was seen. Specimens were submitted to Dr. Ellis.

EXPERIMENT No. XVIII was a futile attempt to determine whether or not Cargile membrane could be made to replace, with any degree of efficiency, the dura mater. The temporal muscles of a dog having been turned down from the side of the head, the skull was opened by trephining. It was intended to remove a portion of the dura and replace it with Cargile membrane. Hæmorrhage, however, was copious, and I contented myself with rolling up a piece of unchromicized Cargile membrane three by four centimetres in dimensions, making a roll the size of a probe. This was wrapped about with several turns of fine silk suture to retain the form, in the hope that I might identify it when again sought. It was simply placed under the flap of temporal muscles to determine the action of the body juices. The wound, however, suppurated and vitiated the experiment, and the membrane was not again seen.

EXPERIMENT No. XIX.—A dog's temporal muscles having been turned down, the skull was trephined, and by means of rongeur forceps an opening in the skull two by three centimetres in dimensions was made, a piece of dura one by two centimetres was turned back and resected. This was replaced by a piece of chromicized Cargile membrane, the edges being slipped well under the dura throughout the entire periphery. A suppurating wound vitiated the experiment; but the resistance of the membrane is shown from the fact that, when removed thirty days later, the membrane was still intact, though porous and brittle. It was submitted to Dr. Ellis.

Two other operations were performed to determine, if possible, the efficacy, if any, of Cargile membrane in the cranial cavity. My results, on the whole, were bad; infection, as a rule, vitiated the experiments, and only the four were tried.

Judging from my work, however, I am inclined to believe from the frailty of the membrane and the difficulty of the handling it, except in the dry state, that the unchromicized variety is without value in cerebral surgery. I am inclined to think better of the chromicized membrane for this purpose. It is much more easily handled in the presence of a moist surface, and is not absorbed for a much longer period.

This completed the series of experiments so far as they seemed of value for the purpose of record.

My estimate of the value of Cargile membrane in preventing adhesions in the situations under consideration is embodied in our joint conclusions at the end of the article.

I avail myself of this opportunity to express my gratitude to Professor Coplin for his interest in this research, and for invaluable laboratory materials placed at my disposal; to Professors Keen and Da Costa for valuable suggestions and material aid; and to senior students C. C. White, L. F. Milliken, and Richard F. Taylor for assistance in the operative work.

HISTOLOGIC STUDY BY DR. ELLIS.

My part in this investigation consisted in studying histologically a number of specimens obtained at operation or autopsy by Dr. Craig. The tissues were fixed in Heidenhain's or Bensley's fluid and finally embedded in paraffin. Sections were stained by hæmatoxylin with the addition of eosin or Van Gieson, Mallory's reticulum stain, polychrome methylene blue, and Weigert's stain for elastic tissue. Those stained by hæmatoxylin and Van Gieson were the most satisfactory for purposes of study. I am deeply indebted to Professor W. M. L. Coplin for advice and assistance during the progress of the work. The description can best be taken up seriatim as the specimens were obtained and according to the experiment numbers of Dr. Craig.

The first specimen studied was a piece of unused Cargile membrane, sections of which were mounted and stained to obtain a basis of comparison for that in the tissues. The infiltrated mem-

brane is very brittle, and in many of the sections is broken into numerous fragments. This must be borne in mind in interpreting the later findings; breaking alone cannot be considered as evidence of actual destruction by the tissues. The membrane elects fibrous tissue stains and by them is colored deeply. The larger part appears homogeneous, but in many areas the membrane seems to be made up of several layers which are intimately fused. For this reason they are not clearly differentiated, but are indistinctly outlined by slight differences in stain reaction. These differences are not sufficiently definite to warrant the assumption that in the preparation of the material it is actually formed by assembling several layers. The membrane contains neither demonstrable cells nor cell nuclei.

EXPERIMENT II.—Intestine on which was placed Cargile membrane under four different conditions; specimen removed at end of fourteen days. A. Peritoneum denuded; chromicized Cargile. Over the operated area the peritoneum and longitudinal muscle are lacking. On the surface of the circular muscle and intimately connected with it is a layer of new fibrous tissue. At either margin of the denuded area, where the Cargile was folded upon itself, are from two to four layers of almost perfectly intact membrane. Between these layers, as well as separating them from the intestine, is new fibrous tissue. At both margins, beyond the Cargile, the omentum is firmly adherent. This new tissue also encloses the portions of the membrane still remaining. The whole area of adhesions thus appears to be surrounding and healing in the layers of membrane. Within the folds of the latter at one margin is a number of so-called foreign body giant cells, some of which are very large. The majority of these cells are in the new tissue at some distance from the membrane, but a certain number are directly upon it. Even where they are in contact with the Cargile, that material shows no evidence of degenerative action due to the cells, and phagocytic action by them is not demonstrable. Between the areas of adhesion at the margins of the denudation, Cargile is present only at some distance from the intestine, and there in the shape of short fragments that show some thinning. That it was broken by the knife in cutting may be inferred from the facts that the new fibrous tissue over the intestine beneath is firm and smooth, and that no adhesions of the omentum have formed. Sections lower in the block, from the

undenuded margin of the described area, show practically the same condition at the borders where adhesions have formed. Between these borders the appearance is also much the same, except that a narrow zone of very loose fibrous tissue is on the surface of the peritoneum; this zone is continuous externally with a band of dense new fibrous tissue similar to that over the denuded area. At points quite broad bands of new tissue extend from the peritoneum across the comparatively clear zone to the superficial layer, and thereby anchor it firmly to the intestine; this attachment of the new tissue, however, is not so intimate as in the case of the exposed muscle in the denuded area. Sections stained by polychrome blue show the presence of a very few cocci arranged singly and in pairs; morphologically they correspond to the ordinary pyogenic cocci.

B. Peritoneum denuded; unchromicized Cargile. The Cargile has essentially the same arrangement as in A. The folds at the margin of the denuded area are more fragmented, and the pieces show more disintegration than in the preceding instance. It is surrounded by the new tissue of omental adhesions. At one margin is the peritoneum and longitudinal muscle of a second coil of intestine that is firmly adherent at this point. Giant cells are not seen.

C. Peritoneum intact; chromicized Cargile. New fibrous tissue has formed on the surface of the peritoneum. The latter structure is dissociated, and through it the new tissue is extending into the outer muscle layer, where it substitutes certain of the fibres. As in the two preceding instances, there are dense omental adhesions beyond the margins of the membrane, and they extend inward and enclose the folds of that material. It is fragmented, conspicuously so between the adhesions where omentum is absent, but otherwise is fairly well preserved.

D. Peritoneum intact; unchromicized Cargile. This specimen is essentially the same as C, in which chromicized membrane was used. The membrane is slightly more frayed on the margins. Where the omental adhesions have formed and included the Cargile, the underlying peritoneum, as such, is no longer clearly demonstrable because of its disruption and intimate association with the new tissue.

EXPERIMENT IV.—Intestine on which was placed Cargile membrane under four different conditions; specimen removed at

end of four days. A. Peritoneum intact; chromicized Cargile. On the surface of the peritoneum of half the circumference of the intestine is a layer of formative tissue covered by fibrin in which is entangled a great many red blood-cells. At some points are numerous polynuclear leucocytes. The peritoneum is infiltrated with leucocytes which also invade the longitudinal muscle. Slight suppuration has occurred on the surface of the exudate as shown by many irregular spaces in the fibrin net-work, some of which contain granular detritus and polynuclear leucocytes. External to the exudate and not intimately attached to it is the Cargile, which is present on the borders of the involved area only, the middle half having almost or entirely disappeared. The appearance of the specimen and comparison of it with similar tissues indicate that the Cargile over the central portion disappeared mechanically during preparation or cutting of the tissue. That part which is present is broken into long pieces, but otherwise is intact. The exudate beneath the membrane and that included in the free central area are identical in structure. There is no evidence of adhesions of any kind.

B. Peritoneum denuded; chromicized Cargile. The peritoneum and longitudinal muscle are lacking. Over the denuded area is a fibrinocellular exudate in which organization is beginning, fibroblastic tissue being present on the surface of the circular muscle, which is infiltrated with leucocytes. Over this exudate is Cargile, which is intact throughout. There is no exudate external to the membrane and no signs of adhesions. Sections from a second block of this specimen are from the undenuded margin of the described area. They differ but little from the denuded space. Organization of the exudate is slightly further advanced. The Cargile over the denuded and undenuded areas presents the same appearance. Sections stained for elastica show none in the newly formed tissue; that in the vessels of the intestine is unchanged.

C. Peritoneum intact; unchromicized Cargile. An exudate composed of fibrin and polynuclear leucocytes is on the surface of the peritoneum, which is also infiltrated with these cells. Organization is beginning in the deeper layers, where vascularized tissue has already formed. The Cargile membrane has entirely disappeared. No adhesions have formed. Giant cells are not present. Sections appropriately stained show the presence of a

very few cocci differing in no way from the ordinary pyogenic types.

D. Peritoneum denuded; unchromicized Cargile. The peritoneum and longitudinal muscle are lacking. The circular muscle is infiltrated with leucocytes. On the surface is a thin layer of vascularized organizing exudate which is surmounted by numerous wavy fibrils of Cargile, appearing as if several sheets of the membrane had split a number of times and the layers had then broken into short fragments. Into the inner portion of this mass of membrane the formative tissue is extending. External to the membrane, for a part of its extent, are fibrin and polynuclear leucocytes. Covering the remainder of the Cargile and also surmounting the fibrinous exudate is a second layer of organizing tissue arising from the omentum, which here is closely adherent (Fig. 1). The serous covering of the omentum is disrupted, and the new tissue extends through it and for some distance into the underlying adiposa. At several points where formative tissue approaches the dissociated Cargile from both sides, the fibroblasts extend directly through the mass of fragments, forming continuous bands which at either end are vascular and becoming distinctly fibrous in character (Fig. 2). Sections from a second block of this specimen are from the undenuded margin. With the exception that the intestinal coats are intact, though infiltrated with the leucocytes, there is no essential difference from the denuded area. The adhesion of the omentum is the same as described when considering the preceding sections. In sections stained by polychrome blue, there are seen in the exudate moderate numbers of cocci arranged singly and in pairs.

Experiment VII.—Intestine of dog from which peritoneum was denuded and Cargile membrane applied; specimen removed at end of three days. Under the microscope, as macroscopically, no membrane is to be seen. The peritoneum is lacking over a considerable area, and, except at the extreme margins, the longitudinal muscle also has been removed. The central part of this denuded area is covered by a thick layer of exudate which is mainly fibrin, but also contains a few leucocytes, both mono- and polynuclear. This fibrinous exudate extends into the circular muscle, separating many of the superficial fibres. Beyond this, for more than half its breadth, the muscle is infiltrated with leucocytes, mainly polynuclears. Near the margin of the denuded

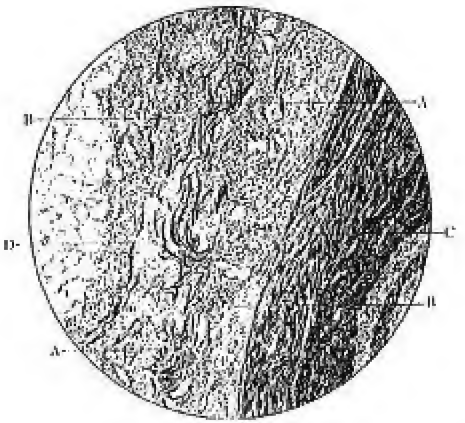

FIG. 1.—Intestine denuded of peritoneum and covered with non-chromicized Cargile membrane. Appearance at end of four days. (B. and L. ⅔ obj., 1 inch ocular.)

A A. Fibrillated and fragmented remains of the Cargile membrane.

B B. Organizing exudate on either side of the membrane. That on the left, especially in the upper part of the field, is still largely fibrinous.

C. Circular muscle of the intestine; at this point the longitudinal muscle was removed during denudation. To the right are the submucosa and basement membrane.

D. Disrupted serosa of the adherent omentum.

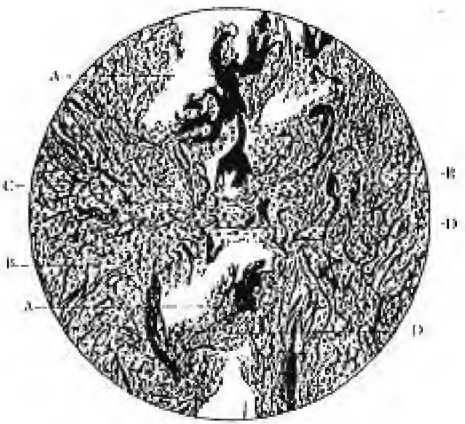

FIG. 2.—From the same section as Fig. 1. (B. and L., ⅙ obj., 1 inch ocular.)

A A. Fragments of the disintegrating Cargile membrane.

B B. Organizing tissue on either side of the membrane. That to the right is on the surface of the intestine; that to the left, on the surface of the adherent omentum.

C. Capillary blood-vessel in the new tissue.

D D. Two areas in which fibroblasts from either side have met through the fragmented membrane, thereby forming bands of adhesion.

area is a great deal of blood. The longitudinal muscle as it appears on the margin is densely infiltrated with leucocytes, and also contains numerous red blood-cells. Blood-vessels of the musculature are distended and contain an excess of leucocytes. The muscle fibres show varying degrees of atrophy. The fibrinous exudate extends for some distance over the peritoneum on either side of the denuded area, gradually thinning as the distance becomes greater. The sections are perfectly free from adhesions to omentum or other surrounding tissue.

EXPERIMENT VIII.—Intestine from which peritoneum was denuded and covered with Cargile membrane, which in turn was covered by rubber dam; specimen removed at end of three days. Microscopic examination of the denuded area shows absence of the peritoneum and essentially all of the longitudinal muscle. On the surface of the circular muscle is a thick layer of exudate which is mainly red blood-cells, but also contains some fibrin and a few leucocytes. This extends into the muscle and separates many of the superficial fibres. The blood-vessels of the muscle are distended, and the inference is that from them hæmorrhage has occurred. Surmounting this mass of blood is a layer of Cargile membrane, which for a small part of its extent, at one end, is perfectly intact. Throughout the greater part of its length it has undergone more or less marked disintegrative changes. It is split into numerous thin layers or fibrils, and these are broken into pieces irregular in shape and of variable size, some being very small. The fragments are widely separated, occupying a space many times as broad as the normal Cargile. Between and surrounding these fragments is the exudate. External to the membrane is a layer of exudate nearly as thick as that between the membrane and the intestine, but differing greatly from it in constitution. The former is made up almost wholly of fibrin and polynuclear leucocytes, very few red blood-cells being present. Leucocytes are exceedingly numerous, and both they and the fibrin show some necrosis. At the point where the Cargile is intact, there is a very sharp differentiation between this external layer of fibrinocellular exudate and the blood beneath the membrane. Where the Cargile is disintegrating, the blood has passed through and permeated for some little distance the exudate externally; the polynuclear leucocytes of the latter have in turn penetrated the blood-clot, this admixture through the partially destroyed mem-

brane being very conspicuous. Polynuclear leucocytes are at many points in direct contact with the fragments of Cargile, but there is no evidence of special disintegration at those places. Phagocytosis is not demonstrable.

EXPERIMENT IX.—Fold of chromicized Cargile membrane that was enclosed in a perforated glass tube and placed in peritoneal cavity; tube removed at end of two weeks. A small amount of reddish-colored material adhered to the end of the tube near the largest opening. Under the microscope this is shown to be made up of red blood-cells and leucocytes, the latter ten times as numerous as the former and mainly polynuclear in type. Eosinophiles are not in greater proportion to other leucocytes than in normal blood. On section, the Cargile is found folded in many layers. The membrane is slightly thicker than normal, or when placed on tissue, and appears to be swollen, possibly by the imbibition of fluid. This appearance is further heightened by lessened density, as shown by the staining reaction and also by roughening or slight fraying of the surfaces. The membrane, however, is intact throughout. Between the layers are masses of partly disintegrated red blood-cells and numerous leucocytes, mainly polynuclear in type.

EXPERIMENT X.—Intestine denuded of peritoneum and covered by Cargile membrane, the latter being covered by rubber dam; specimen removed at end of six days. The peritoneum and longitudinal muscle are lacking. On the surface of the circular muscle is a thick layer of organizing exudate, the most advanced portions of which, bordering the muscle, are just assuming the characters of fibrous tissue; external to this is a well-marked zone of vascularized tissue, and on the surface a layer of fibrin and polynuclear leucocytes. The circular muscle also shows leucocytic invasion. In the fibrinous exudate at one point are a few fragments of Cargile membrane, the remainder having entirely disappeared.

EXPERIMENT XI.—Cargile membrane from a sealed celloidin capsule that was in the peritoneal cavity for seven days. The membrane is very much swollen, most of it being more than twice the normal thickness. The margins are decidedly frayed, presenting at some points a serrated appearance. Although the sections are very thick, the density is much lessened, many areas being semi-translucent; at points are small clear spaces or open-

ings. Stains are taken with much less avidity than by the other specimens of the membrane studied. No cells are present. The appearance of this specimen is strongly indicative that the membrane is undergoing slow absorptive changes.

EXPERIMENT XII.—Cargile membrane and fluid from celloidin capsule that had been in peritoneal cavity thirty days. This specimen was first examined by Dr. Coplin, who kindly furnished the following description: "The capsule is surrounded by what appears to be fibro-fatty tissue, presumably a part of the omentum. Around the irregular and slightly rough end of the capsule, that had been closed by ligature and sealing, the tissue attains a thickness of two to five millimetres. Towards the opposite or smooth end of the capsule the enveloping tissue hardly exceeds one millimetre, and at points is so thin that it is quite transparent. After incision of the soft tissue the capsule readily slipped out. Along one side it is dark in color, and in places is slightly wrinkled. It is evident there is fluid within, but it escapes at no point, even when gentle pressure is made upon the capsule. Upon opening the latter, the contained fluid is found to be of about the consistency of blood serum, slightly opalescent, possessing a faint pink tinge, decidedly cloudy, and containing scarcely perceptible irregular granules to which the cloudiness appears to be due. This fluid was examined in the fresh condition, also stained by Sudan III, methylene blue alone, and with eosin, and by Wright's stain. It is found to contain large quantities of granular material of a form usually characterized as cellular detritus. Some of the granules are grouped, and occasionally small, stringy granular bodies are observed. The granules vary in dimensions from one to four or five microns, and in some fields are collected into masses 100 or more microns in diameter. The larger number of granules are strongly acidophilic. With them are numerous spherical bodies possessing the general appearance of fat droplets and taking Sudan III strongly. Occasionally one sees what, by stretching the imagination, may be thought to resemble a shrunken cell of some kind; such bodies, however, are extremely rare. No structures resembling leucocytes or bodies corresponding to any histological structure can be identified. By proper staining methods, bacilli two microns in length and less than one micron in width are seen to be fairly abundant. These bodies could be recognized in unstained specimens, and were not motile. Cocci of ordinary

dimensions, indistinguishable from usual pyogenic organisms of this group, are occasionally observed; they are not, however, in masses, nor are they abundant. The bacilli are far more numerous. The bacteria were not identified. The capsule also contains an extremely thin membrane-like structure, the dimensions of which are not determined." Later examination of this structure left little doubt that it was the much thinned Cargile membrane. It was left in salt solution for some hours; at the end of that time the salt solution was very turbid and the membrane had entirely disintegrated and disappeared. The value of this experiment, undertaken to determine the effect upon Cargile membrane of body fluids without the presence of cells, was vitiated by the occurrence of infection, and deductions therefore must be restricted.

The new tissue which had formed around the capsule is a band of varying breadth, the external portion of which is quite dense, newly formed fibrous tissue. Firmly adherent to three-fourths of the circumference is normal appearing adipose tissue. Towards the inner surface of the band, the fibrous tissue is less dense, and contains more cells. On this surface at points are leucocytes, both mono- and polynuclear in type. At other places, or along with the cells, is considerable fibrin. Both cells and fibrin show evidence of slight necrosis.

Experiment XIII.—Posterior tibial nerve which was isolated and wrapped with Cargile membrane; specimen removed at end of fourteen days. A. Nerve from left side, covered with four layers of unchromicized Cargile. Sections from one block of this specimen show between the nerve trunk and the fibrous tissue which half surrounds it the layers of the membrane. Of the four layers, the outer two, or those in contact with the tissues on either side, are intact, or nearly so. The two inner layers are not so well preserved. All four are separated some distance from each other in the wide space between the nerve and the enclosing tissue. The outer layers are partly enclosed by polyblasts or by recent fibrous tissue. This extends through the small breaks that are present in the membrane. Organizing tissue is also found between the layers of the membrane, but is not so prominent around the two inner as is that enclosing the two outer. No distinct adhesions are present in this section, the newly forming fibrous tissue on the two sides apparently being prevented by the membrane from

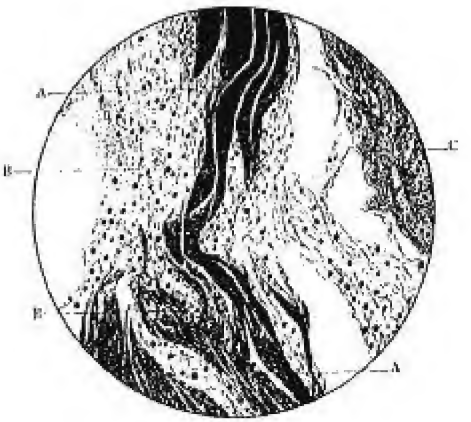

FIG. 3.—Layer of non-chromicized Cargile membrane surrounding posterior tibial nerve for fourteen days. (B. and L., $\frac{1}{12}$ homo. imm., 1 inch ocular.)

A A. Cargile membrane splitting into fibrils, but otherwise fairly well preserved.

B B. Spindle-shaped fibroblasts which are entering between the fibrils of the membrane. The appearance at and below the lower letter indicates that there is an intimate connection between the splitting of the Cargile and the intercalation of the formative cells.

C. New fibrous tissue internal to the membrane.

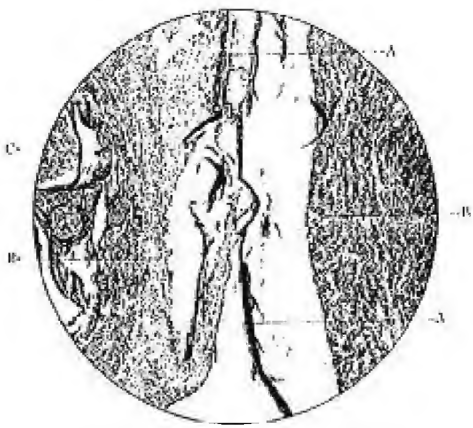

FIG. 4.—Three layers of chromicized Cargile membrane surrounding posterior tibial nerve for fourteen days. (B. and L., $\frac{2}{3}$ obj., 1 inch ocular.)

A A.—Cargile membrane. Two layers and a few isolated fragments of the third are still present. Varying degrees of fibrillation and fragmentation are shown.

B B. Newly formed or forming fibrous tissue bordering the space containing the Cargile. That on the right surmounts the connective tissue separated from the nerve when the membrane was placed; on the comparatively smooth border repair is sufficiently advanced probably to be beyond the adhesive stage. The new tissue on the left, surrounding the isolated nerve, is not so far advanced; on the surface and extending between the layers of the membrane is fibrinous exudate containing a few leucocytes.

C. Part of a nerve bundle immediately beneath the new tissue. Degenerative changes have rendered this portion of the nerve almost unrecognizable.

uniting. A few giant cells are in the new tissue surrounding the nerve. One large one with six nuclei has in it a fragment of fibrous tissue that is roughened, and appears not unlike equal-sized pieces of Cargile membrane as it is found elsewhere. From the fact that these are typical "foreign body" giant cells developed only in the neighborhood of the membrane, it is reasonable to suppose the Cargile is the origin of the fragment in question. Whether or not this be an instance of phagocytic destruction and removal of the membrane, it is the only suggestion of such process found in the entire series of specimens. The membrane in those areas where reparative processes are most active is splitting into fibrils, and between them polyblasts and spindle-shaped fibroblasts are insinuating their way (Fig. 3). In this manner the membrane appears to be disrupted and removed, or finally incorporated with the new tissue. Sections from another block of this specimen show the new fibrous tissue more prominently; at one point is a continuous band joining the two sides, though it extends in an irregular and zigzag manner among the fragments of Cargile. The appearance of the entire section is that uniform adhesions will finally result. A few giant cells are present, but they are not large, and are not in direct contact with the membrane.

B. Nerve from right side, covered with three layers of chromicized Cargile. Two layers of Cargile extend entirely around the nerve, except where broken in cutting or by destructive action of the tissues. Within these, directly upon the nerve, is a band of forming fibrous tissue, upon the surface of which is a fibrinous exudate containing many red blood-cells; this exudate is for the most part in contact with the Cargile. The areolar connective tissue, which was separated from the nerve when the Cargile was placed, is also covered by a layer of new tissue which is smooth and sharply limited as though repair was complete; it is nowhere penetrating or adherent to the membrane in the sections from A (Fig. 4). No giant cells are seen. Sections from another block of this specimen show new tissue advancing between the layers of Cargile, but no adhesions have formed.

EXPERIMENT XIV.—Tendo-Achillis and posterior tibial nerve. Two layers of Cargile around tendon, nerve not covered; specimen removed at end of twenty days. Cargile can be identified over approximately three-fourths of the circumference of the tendon. It is split into several thin layers and broken into short

fragments. Throughout the entire extent, where visible, it is enclosed in a narrow space bounded by dense, newly formed fibrous tissue. For a part of the distance it is partially free in this space, which also contains red blood-cells. In such areas actual adhesions do not appear to have formed. At irregular intervals, however, fibrous bands unite the tissue on either side, and the Cargile is thus incorporated in a nearly healed wound; at many of these points the membrane has essentially lost its identity as a distinct structure. In several areas are numerous foreign body giant cells nested in small spaces, which they entirely fill or they are surrounded by loose areolar tissue. From these areas the Cargile has entirely disappeared. Phagocytosis by these cells is not demonstrable. In the fourth of the circumference where Cargile is entirely absent is a solid band of fibrous tissue, giving the impression that the membrane had not been present over this area.

EXPERIMENT XVII.—Tendo-Achillis and posterior tibial nerve. These were separated and each covered with Cargile membrane; specimen removed at the end of fifty-four days. In sections from this specimen can be found no evidence whatever of the membrane or the place formerly occupied by it. There appears to be but little excess of fibrous tissue over that which would normally be found in this location. At one point is a small circumscribed area made up almost entirely of giant cells surrounding fragments of a suture.

EXPERIMENT XIX.—Chromicized Cargile membrane from brain of dog; specimen removed at end of thirty days. This specimen is very brittle when mounted. Sections show that portions are of normal density, but slightly thinned. Still other parts are thickened, spongy in character, and stain less deeply than usual. The total bulk of the membrane appears to be slightly less than for normal membrane of the same extent; the loss, however, is not conspicuous.

The object of these histologic studies was to determine, if possible, the fate of Cargile membrane in the tissues, and also its effect upon those tissues it was intended to protect. The major portion of the findings has been embodied in our conclusions, but one point seems worthy of special emphasis. The irritative action of the membrane as a foreign body,

especially in the peritoneal cavity, is so pronounced that it cannot be disregarded, and appears to be the principal factor militating against the otherwise beneficent possibilities of the material. In the case of raw surfaces it is difficult to estimate this action, but in every instance in which the membrane was placed over intact peritoneum, reactionary new tissue formed on the surface of the latter, which in many cases was disrupted and incorporated with the new formation. When the membrane is placed between two freshly incised surfaces, this stimulus towards "healing in" of the foreign material is added to the reparative efforts common to all wounds, and their resultant action must be withstood if adhesions are prevented. It does not appear that Cargile membrane is able so to do.

Our joint conclusions are:

1. The most distant time at which we found unchromicized Cargile membrane existing intact, macroscopically, within the peritoneal cavity, was the fourteenth day; in most instances it had disappeared to macroscopic view much sooner. The earliest time at which we found the membrane had disappeared over the area of actual denudation was on the third day.

2. Unchromicized Cargile membrane when buried in living animal tissue, as when placed around tendons and nerves, or in muscle, is apparently absorbed sooner than when placed within the peritoneal cavity. In no instance was so much as a fragment of the membrane observed macroscopically so late as the fifth day, though in the fragmental state membrane was noted microscopically so late as the fourteenth day.

3. Chromicized Cargile membrane when placed within the peritoneal cavity or when buried in living animal tissue remains unabsorbed much longer than does the unchromicized variety. The two varieties doubtless bear relatively the same relation to each other, so far as absorbability is concerned, as do chromicized and unchromicized catgut.

4. While the unchromicized, and to a less extent the chromicized, variety will adhere fairly firmly to a surface denuded of peritoneum when such surface is relatively dry, yet neither can be depended upon to remain where placed, unless

anchored by some method, in a situation which is subject to peristaltic activity.

5. A logical deduction from the results of the foregoing experiments seems to warrant the belief that neither variety of the membrane is of value in preventing adhesions within the peritoneal cavity. In every instance the membrane, until absorbed, appeared to act as a foreign body, and therefore as an irritant.

6. We believe from the results of our observations that both varieties of the membrane are of value in preventing adhesions to wounded nerves and tendons when such structures lie in tissues which have been subjected to trauma, operative or otherwise. Our conviction is that for this purpose the chromicized is the more valuable.

7. We believe that several layers of either variety of the membrane when placed around tendons or nerves afford a safer and better protection than one layer.

8. We believe that, when used in the cranial cavity to replace destroyed or removed dura, the unchromicized variety would be exceedingly difficult to handle on account of its being unmanageable when moist; and we further believe, on account of the rapidity with which it dissolves, that it would be of no special value in this situation even though it could be used with ease. Owing to the facility with which the chromicized variety can be handled, its greater toughness and increased power to resist absorption, we believe that it would prove of greater value in replacing the dura.

9. Our studies indicate that the membrane is destroyed by a lytic substance, or substances, contained in the body fluid. The celloidin capsule experiments, even though bacteria were present in one, show that the membrane is softened, and at least partially absorbed by body fluids without the presence of cells. In the tissues it is split into fibrils, this change being accompanied or followed by the penetration of formative cells of the new tissue enclosing it. Fragmentation, disintegration, and absorption finally ensue. Phagocytosis may safely be excluded as a chief important contributing cause.